Applicants initially note that the Examiner considers that claims 24-33 "are withdrawn from consideration". The Applicants respectfully submit that claims 32 and 33, filed on February 21, 2001 when responding to the requirement for restriction and which are method claims for modulating Annexin-based MDR, are pending in the application. Thus, the Applicants respectfully submit that prior to the present response, claims 15-23 and 32-33 were pending in the application.

Oath/Declaration

An Oath/Declaration is filed herewith, in view of replacing the one previously filed which was deemed "difficult to read".

Abstract

The abstract on a separate page is also herewith submitted. This abstract is identical to the one which was initially filed upon filing of the PCT application on which the instant application is based.

Amendment of Claims

The claims have been amended in view of the Official Action. Support for the amendments to the claims can be found throughout the specification as originally filed. Further support for the terminology "cytotoxic" can be found, for example, at page 2, line 1; at page 20, line

21 and at page 25, line 14. The amendments to the claims are mostly of editorial nature and aimed at better defining what the Applicants consider their invention.

Rejection under 35 U.S.C. § 112, Second Paragraph

The Examiner has rejected claims 15-23 under 35 U.S.C. § 112, second paragraph for "failing to distinctly point out the subject-matter Applicant regards as his invention". The Applicants traverse the rejection as follows.

First, the Applicants respectfully submit that the claims have been amended to more particularly point and distinctly claim the subject-matter which the Applicants regard as their invention. More particularly, the terms "effects" and "effecting" have been canceled. "MDR" has been defined, at least in its first instance, by "multidrug resistance".

The objection with respect to the lack of clarity of how a compound could be selected has been rendered moot by the amendments to claim 15.

Applicants also submit that the alleged lack of clarity of the term "drug" has been corrected. The method steps enabling a demonstration on how to assess the effect of the compound has been further defined.

With respect to the terminology "small molecule" in claims 17 and 20, the Applicants respectfully submit that

this well-known terminology is clear to a person of ordinary skill in the art. In addition, the Examiner is referred to page 30, lines 3-7 which relate to "small molecule".

With respect to the allegation of vagueness and indefiniteness for the term "an antisense", it is believed that in view of the amendment which now relates to an antisense nucleic acid, and to the legend of Figure 1, which exemplifies antisense nucleic acid molecules which can be used in accordance with the present invention, that claims 18 and 22 should be considered clear and definite by a person of ordinary skill.

Finally, the Examiner has rejected claim 20 because it is alleged that the term "modulating" is indefinite because it "does not recite whether modulation is up or down". The Applicants respectfully submit that indeed, the term "modulation" is used herein to cover both an increase in resistance and a decrease in resistance of a cell to Annexin-based MDR. The bi-directional nature of this modulation is clearly supported throughout the disclosure and it is believed that the recitation is clear and distinct.

In view of the above and foregoing, withdrawal of the rejections under 35 U.S.C. § 112, second paragraph is respectfully solicited.

Rejection Under 35 U.S.C. § 102(b)

The Examiner has rejected claims 15 and 19-20 under 35 U.S.C. § 102(b), as being anticipated by Wang, et al. The Examiner alleges that

> "Wang disclose a method that was used to isolate a monoclonal antibody (IPM96) which recognized a protein (P-40) co-expressed with P-glycoprotein in several resistant cell lines. Wang further discloses that overexpression of P-40 in multidrug resistant cells **may** be important in the expression of the drug resistance phenotype". [emphasis added]

As noted by the Examiner, Wang further

> "...supports the notion that P-40 alone **may** confer resistance to cytototix drugs (see page 486). Wang also disclose that P-40 **could** modulate an MDR phenotype **indirectly**..." [emphasis added].

The Examiner then concludes that

> "Wang anticipates the claimed invention as Wang identifies a compound (P-40) that affects Annexin-based MDR in a cell in the presence of a drug (Adriamycin and Taxol) and assessed the effect of said compound as claimed in the present application." [emphasis added]

The Applicants respectfully traverse the rejection as follows. Firstly, Wang, et al. do not teach that P-40 is directly involved in multidrug resistance in cells. Indeed, as viewed above, only mere speculations of the role of P-40 in MDR can be found. The fact that P-40 has a direct role in MDR was a mere speculation in Wang can be found also in their conclusions at page 487, right column which states:

> "Although further studies are required to demonstrate **a direct role for P-40, if any, in**

> **drug metabolism and MDR**; P-40 **could** modulate an MDR phenotype **indirectly**. For example, P-40 **may** be a component of the apoptosis signaling pathway." [emphasis added]

Clearly, Wang does not teach that P-40 modulates or triggers multidrug resistance. At best, it suggests that it could be a factor which is associated with a multidrug resistant phenotype. The physiology and genetic pattern of expression of genes (including genome amplification, increase in transcription, increase in translation, and differential post-translational modifications) is so complex and unstable in multidrug resistant cells that the recognition that differentially expressed proteins, elevated level of an mRNA, or differential post-translational modification of a protein, for example, reflect a causative event of MDR, as opposed to a consequence of the MDR, can only be assessed by direct analysis of the role of a putative factor and its effect on drug sensitivity/resistance of cells.

As very well known to the person of skill to which the present invention pertains, upon treatment of cells with anti-cancer drugs, one of the mechanisms of survival of the cells which can lead to the MDR phenotype, involves amplification of sequences, thereby creating amplicons of a number of genes. These amplicons give rise to numerous sequences which are co-amplified together with the gene which is responsible for the MDR phenotype (such as P-glycoprotein, an efflux pump which

pumps the anti-cancer drug outside of the cell--the over-amplification of DNA encoding such efflux pumps translates into an increase in the pumping out of the drug, enabling survival of the cell to a much higher concentration of drug as compared to a control cell). In addition to the creation of amplicons, chromosomal breakage due to the mutagenic effect of the anticancer drug used (e.g. DNA intercalating agents and the like) can lead to the juxtaposition of a gene involved in the production of the MDR phenotype and a strong promoter, once again enabling an overexpression of this gene (e.g. efflux pump).

In addition, it is established by the person of ordinary skill, that the majority of amplified genes identified in drug-selected cells have nothing to do with the pathway leading to MDR, but rather reflect events occurring downstream of MDR. In other words, the overexpression reflects a consequence of the MDR phenotype and the aberrant genomic expression patterns in the drug selected cells, rather than a cause of MDR.

It should also be recognized that the fact that a sequence is over-expressed or that a protein is post-translationally modified in drug-selected cells or more broadly that there is an association between expression of a gene or protein and MDR, does not and should not be recognized as more than a mere suggestion that this over-

expression or differential post-translation modification thereof has relevance to the multidrug resistance phenotype *per se*.

The Applicant respectfully submits that the instant application is the first study which provides a teaching of a **direct correlation** between the over-expression of Annexin I (P40) and multidrug resistance. Prior to the present invention, there had been no direct experimental evidence demonstrating that the overexpression of a single gene product by gene transfer into drug sensitive cells could confer resistance to the multiple anticancer drugs (e.g. overexpression of P-40/Annexin I). Indeed, it is clearly recognized by the person of ordinary skill in the art that differential expression of a given protein between drug-sensitive and -resistant cells alone cannot and should not be regarded as a reasonable evidence for the role of such a protein in drug resistance. Such a differential expression can merely be suggestive of a role in MDR. In fact, it more than likely reflects a consequence rather than a cause of MDR (see below).

Numerous examples of proteins have been found to be differentially expressed in drug-selected cells and yet do not mediate or confer drug resistance to drug-sensitive cells upon transfer thereinto (Kool, et al., 1999; Schinkel, et al., 1991; Sugimoto, et al., 1993; a

copy of which are enclosed herewith for the Examiner's convenience). For example, the overexpression of an 85 kDa membrane protein in a resistant sub-line of human myelogenous leukemia K562/ADM was shown by gene transfer studies **not** to mediate drug resistance (Sugimoto, et al., 1993). Similarly, a highly conserved member of the P-glycoprotein gene family, the mdr3 or P-glycoprotein 3, which is amplified in MDR cells was shown by gene transfer studies **not** to mediate drug resistance upon transfection into drug sensitive cells (Schinkel, et al., 1991). More recently, a member of the Multidrug Resistance Protein (MRP), mrp6, which is amplified in MDR cells was also shown **not** to play a role in the resistance of tumor cells (Kool, et al., 1999). Thus, it has long been recognized by the ordinary skilled artisans in the field of multidrug resistance, that in order to establish that a gene and the protein it encodes is involved in multidrug resistance, gene transfer studies have to be performed. In other words, the identification of overexpressed sequences, differentially expressed proteins or genes, or differentially post-translationally modified proteins, should not be viewed as more than merely suggesting a role therefor in multidrug resistance (especially in view of the fact that a majority of such differential modifications are later shown not to be involved in MDR).

Taken together, it is well accepted and established in the MDR field that the mere demonstration of differential expression of a given DNA, RNA, protein, or modification of this protein does not constitute a reasonable evidence for a role in drug resistance. In addition to the above examples, several studies have clearly demonstrated that overexpression of certain gene products result from the co-amplification of various genes with P-glycoprotein and have no role in MDR (Van der Bleik, et al., 1986).

Thus, it is respectfully submitted that Wang, et al. neither teaches nor suggests the direct role of Annexin in multidrug resistance and therefore, in view of the above and foregoing, withdrawal of the rejection under 35 U.S.C. § 102(b) is respectfully solicited.

## CONCLUSIONS

The rejections of claims 15-23 and 32-33 are believed to have been overcome by the present remarks and by the amendments to the claims. From the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order, and such an action is earnestly solicited.

In the event that there are any questions concerning the Amendment, or application in general, the Examiner is

respectfully urged to telephone the undersigned so that prosecution of the application may be expedited.

Authorization is hereby given to charge Deposit Account No. 17-0055 for any deficiencies or overages in connection with this Response.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached pages are captioned "**Version with markings to show changes made**".

Respectfully submitted,

Ellias Georges, et al.

November 5, 2001

By: Jean C. Baker

Jean C. BAKER
QUARLES & BRADY LLP
411 East Wisconsin Avenue
Milwaukee, WI 53202
Registration No. 35,433
(414) 277-5709

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | |
|---|---|
| Applicants: | Ellias Georges, et al. |
| Serial No.: | 09/529,925 |
| Filed: | 07/30/00 |
| For: | P40/ANNEXIN 1 AND RELATED PROTEINS AND THEIR ROLE IN MULTIDRUG RESISTANCE |
| Group Art Unit | 1653 |
| Examiner: | H. Robinson |

---

**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

---

In the Specification:

**ABSTRACT OF THE DISCLOSURE**

The present invention relates in general to multidrug resistance (MDR) in cells. In particular, the present invention relates to the identification of a new member of the MDR gene family, P-40, as well as to the identification of P-40 related genes (homologs) as being additional members of the MDR gene family. The present invention therefore relates to nucleic acid molecules encoding P-40 protein and P-40 protein homologs, to multidrug resistant cells containing these nucleic acid molecules; to hybridomas containing antibodies to P-40 and P-40 homologs; to nucleic acid probes for the detection of these nucleic acid molecules; to a method of detection of such nucleic acid molecules or of the P-40 protein or P-40 homologs; to bioassays comprising the nucleic acid molecules encoding P-40 or P-40 homologs, P-

40 protein or P-40 protein homologs, or antibodies of the present invention to diagnose, assess or prognose MDR in an animal; to therapeutic uses of the nucleic acid molecules of the present invention; and to methods of preventing MDR in an animal.

In the Claims:

Claims 15, 18, 19, 22 and 23 have been amended as follows: Underlines indicate insertions and [ ] indicate deletions.

15. (Amended) A method of identifying a compound that [affects] modulates Annexin-based multidrug resistance (MDR) in a cell, [said method] comprising:

a) incubating said cell in the presence of a [potential] candidate [Annexin-based MDR-affecting] compound in the presence [and] or absence of a cytotoxic drug; and

b) assessing the effect of said candidate compound on the resistance of said cell to said cytotoxic drug;

wherein a candidate compound is selected as a modulator of Annexin-based MDR, when the resistance of said cell to said cytotoxic drug is measurably different in the presence of said compound as compared to the resistance in the absence of the compound.

18. (Amended) The method of claim 17, wherein said candidate compound is an Annexin I antisense [molecule] nucleic acid.

19. (Amended) The method of claim 15, wherein said cytotoxic drug is an anticancer drug.

22. (Amended) The method of claim [21] 35, wherein said compound is an Annexin I antisense [molecule] nucleic acid.

23. (Amended) The method of claim [21] 35, wherein said compound is a calcium chelator or a calcium channel blocker.